

Ovicidal and larvicidal activities of *Cyperus giganteus* Vahl and *Cyperus rotundus* Linn. essential oils against *Aedes albopictus* (Skuse)

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Abstract

The ovicidal and larvicidal efficacy of essential oils extracted from the tubers of *Cyperus giganteus* Vahl and *Cyperus rotundus* Linn. was studied on eggs and fourth instar larvae of *Aedes albopictus* (Skuse). The eggs and larvae were exposed to serial concentration of the oils ranging from 5-150 ppm and kept under observation for 24 h. Both the oils showed remarkable ovicidal and larvicidal activities indicated by EC₅₀ values of <5 ppm and LC₅₀ and LC₉₀ values of <20 ppm. The results obtained suggest that the essential oils of these *Cyperus* species can serve as a potential source of natural mosquitocidal agents.

Keywords: Essential oil, *Cyperus giganteus*, *Cyperus rotundus*, *Aedes albopictus*, Mosquitocide, Ovicidal, Larvicidal.

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Introduction

The "Asian tiger mosquito" *Aedes albopictus* (Skuse) (Diptera: Culicidae) is an important vector of dengue, chikungunya and other viruses¹⁻⁴. Over the last two decades, *A. albopictus* has spread throughout the world, making it currently the most invasive mosquito in the world⁵. In the absence of dengue and chikungunya vaccines, control of this vector appears to be a better strategy for combating these dreadful diseases. Currently, synthetic insecticides are being widely used as major control measure against mosquitoes. The tendency for recrudescence by mosquitoes and diseases caused by them are attributed to the increasing level of resistance developed by them to the commercial synthetic

insecticides⁶⁻⁷. Apart from these facts, indiscriminate use of synthetic insecticides affects the environment and non-target organisms adversely. Targeting breeding sites of mosquitoes has been rated as one of the successful means of regulation of their population density in the field⁸. Utilization of essential oils from plants against insect pests is being evolved as a novel alternative approach to synthetic chemicals in pest control strategies⁹. Essential oils of numerous plants have been shown to possess larvicidal activity against mosquitoes⁹⁻¹¹. The present work aims at investigating the ovicidal and larvicidal activities of the essential oils from rhizomes of *Cyperus giganteus* Vahl and *Cyperus rotundus* Linn. against *A. albopictus* with a long term

intention of the control of recurrent chikungunya and dengue epidemics, through effective vector control strategy. Some of the species of Cyperaceae have attained the status of worst and invasive weeds in many parts of the world^{12, 13}. In India and South-east Asian countries they are known for their medicinal uses. Therefore, it appears more feasible to use these species for economical purpose such as source of medicines and insecticides for the management of these plants in places where they attained the status of weeds. However, knowledge on insecticidal property of Cyperaceae is much limited^{14,18}.

Materials and Methods

Extraction of essential oils

The rhizomes of *C. giganteus* were collected from a pond near Biotechnology Research Centre and that of *C. rotundus* were purchased from a commercial supplier. The samples are deposited in the laboratory. The plant materials were washed with tap water, shade dried at room temperature for 5 days and powdered in a blender. Essential oil was extracted from the dried powdered material through hydro-

distillation. The obtained oil was dried over anhydrous sodium sulfate, collected and stored in light-protected bottles at 4°C until further use.

Preparation of stocks and concentrates

A 1% (v/v) stock solution was always prepared fresh by adding the essential oils to ethanol. Measured volume of the stock was added to 100 ml of tap water to get concentration ranging from 5 - 150 ppm.

Bioassay

A wild colony of *A. albopictus* reared in the laboratory was used for the experiments. The larvae maintained in cages of dimension, 30×60×30 cm³ (Fig.1) at 27±2°C, under 14L:10D photoperiod cycles and allowed to emerge as adults. The males were fed with sucrose solution soaked on cotton pads and females were fed on mice blood every alternate day. Beakers containing wet filter papers were introduced into cages for oviposition. Eggs laid were dehydrated for 15 days and were introduced into water for hatching. Eggs and larvae produced by the colony were subjected for bioassay on ovicidal and larvicidal activity of the test sample.

Ovicidal activity

The Bioassay method by Prajapati *et al*¹⁶ was followed with some modification. Twenty five dehydrated eggs of *A. albopictus* were exposed to five concentrations (5.0, 10.0, 50.0, 100.0 and 150.0 ppm) of essential oils of *C. giganteus* and *C. rotundus* in 25 ml beakers containing 5.0ml of test solution of respective concentration. Trials

on each concentration were carried out in triplicates. Tap water with ethanol (150 ppm) served as control. Viability of the eggs was estimated by determining the ratio of the number of larvae that emerged from the eggs after 24h of treatment in the test solution to that of the total number of eggs subjected for treatment. At the end of 24h of exposure to the test samples, unhatched eggs were manually opened using needle and observed under a microscope (20X) to verify the status of the individual inside.

Larvicidal activity¹⁹

The larvae were fed with yeast and rat chow at 1:1 ratio. Twenty five numbers of fourth instar larvae of *A. albopictus* were kept in 250 ml glass beaker containing 100 ml of tap water with different concentrations (5.0, 10.0, 20.0, 40.0, and 60.0 ppm) of essential oils. Three replicates for each concentration were set up. A control was set up with 20 ppm of ethanol in 100 ml of tap water.

Statistical analysis of data

Results of the above bioassay were subjected for analysis using computerized Probit analysis (StatsDirect), to calculate EC₅₀, LC₅₀ and LC₉₀ values along with Chi² test for assessing the ovicidal and larvicidal activities of test samples. Regression equations of these data are provided in the results and discussion.

Results and Discussion

Ovicidal effect of the test samples on eggs of *A. albopictus* has been illustrated in Fig. 2. The picture shows the dead larvae (which were unable to emerge out of the eggs as seen under the

microscope) on manual opening of the egg case in the laboratory and a normally hatched larva. Results of the ovicidal bioassay of *C. giganteus* and *C. rotundus* oils on *A. albopictus* are presented in Table 1. From the table it is evident that oils of both the plants have effective ovicidal activity at minute concentration levels with EC₅₀ values of 2.8 ppm, with a regression equation of $y = 0.050x + 18.281$; $r^2 = 0.643$ in the case of *C. giganteus* and 4.2 ppm with regression equation of $y = 0.065x + 16.716$; $r^2 = 0.658$ for *C. rotundus*. Results of larvicidal assay (Table 2) also showed similar trend exhibiting effective larval mortality with LC₅₀ and LC₉₀ values of 9.8 ppm and 13.3 ppm ($y = 0.242x + 10.210$; $r^2 = 0.990$) for *C. giganteus* and 12.2 ppm and 18.8 ppm ($y = 0.288x + 7.157$; $r^2 = 0.957$) for *C. rotundus*. From the above results it is obvious that *C. giganteus* possesses more powerful biocidal effect on the target organism than *C. rotundus* as evident through the lower EC₅₀, LC₅₀ and LC₉₀ values. One of the major components (α -cyperone) of the essential oil of *C. rotundus*^{20, 21} has been attributed for its insecticidal activity on moths and beetles^{17, 22}. However, presence of α -cyperone has not been reported from essential oil of *C. rotundus*²³. In the light of the current result on biocidal parameters tested on *A. albopictus*, it is reasonable to presume the involvement of some other component(s) also in imparting insecticidal property to these oils. Repeated bioassay coupled with analytical fractionation of the oil component is needed to confirm the actual compound(s) responsible for insecticidal property of the oils.



Fig. 1: Mosquito rearing cage



Fig. 2 : Manually opened eggs showing dead larvae along with a normally hatched larvae

Table 1: Ovicidal activity of essential oils of *Cyperus giganteus* and *Cyperus rotundus* against eggs of *Aedes albopictus*

Plant species	Conc. in ppm	Mortality%	EC ₅₀	95% CL (in ppm)		Chi ² test
				LCL EC ₅₀	UCL EC ₅₀	
<i>C. giganteus</i>	5	60.0	2.8	0.3	5.4	19.27*
	10	84.0				
	50	90.6				
	100	94.6				
	150	100.0				
	Control	8.0				
<i>C. rotundus</i>	5	50.6	4.2	1.5	7.8	21.20*
	10	80.0				
	50	88.0				
	100	98.6				
	150	100.0				
	Control	8.0				

PPM: Parts per million; LC: Lethal concentration; CL: Confidence limit; LCL: Lower confidence limit; ULC: Upper confidence limit.
*Significant at $P < 0.05$ level.

Conclusion

The present study has revealed the potential of Cyperaceae as a source of mosquitocidal compounds and reports the insecticidal property of *C. giganteus* for the first time.

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Table 2 : Larvicidal activity of essential oils of *Cyperus giganteus* and *Cyperus rotundus* against 4th instar larvae of *Aedes albopictus*

Plant species	Conc. in ppm	Mortality%		LC ₅₀ (LC ₉₀)	95% CL (in ppm)	Chi ² test
		LCL LC ₅₀ (LC ₉₀)	UCL LC ₅₀ (LC ₉₀)			
<i>C. giganteus</i>	5	1.3	9.8 (13.3)	8.4 (11.5)	11.2 (25.2)	70.52**
	10	52.0				
	20	100.0				
	40	100.0				
	60	100.0				
	Control	0.0				
<i>C. rotundus</i>	5	4.0	12.2 (18.8)	6.1 (6.2)	24.5 (67.9)	53.80**
	10	20.0				
	20	100.0				
	40	100.0				
	60	00.0				
	Control	0.0				

PPM: Parts per million; LC: Lethal concentration; CL: Confidence limit; LCL: Lower confidence limit; ULC: Upper confidence limit; IC₅₀: *C. giganteus*; IC₉₀: *C. rotundus*
 ** Significant at $P < 0.0001$ level.

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